Regulation and role of PDGF receptor α-subunit expression during embryogenesis

GINA C. SCHATTEMAN1, KATHLEEN MORRISON-GRAHAM2, ANJIE VAN KOPPEN1, JAMES A. WESTON2 and DANIEL F. BOWEN-POPE1.*

1Department of Pathology, University of Washington, Seattle, WA 98195, USA
2Institute of Neuroscience, University of Oregon, Eugene, OR 97403, USA

*To whom correspondence should be addressed

Summary

The platelet-derived growth factor receptor α-subunit (PDGFRα) is the form of the PDGF receptor that is required for binding of PDGF A-chain. Expression of PDGFRα within the early embryo is first detected as the mesoderm forms, and remains characteristic of many mesodermal derivatives during later development. By 9.5 days of development, embryos homozygous for the Patch mutation (a deletion of the PDGFRα) display obvious growth retardation and deficiencies in mesodermal structures, resulting in the death of more than half of these embryos. Mutant embryos that survive this first critical period are viable until a new set of defects become apparent in most connective tissues. For example, the skin is missing the dermis and connective tissue components are reduced in many organs. By this stage, expression of PDGFRα mRNA is also found in neural crest-derived mesenchyme, and late embryonic defects are associated with both mesodermal and neural crest derivatives. Except for the neural crest, the lens and choroid plexus, PDGFRα mRNA is not detected in ectodermal derivatives until late in development in the central nervous system. Expression is not detected in any embryonic endodermal derivative at any stage of development. These results demonstrate that PDGFRα is differentially expressed during development and that this expression is necessary for the development of specific tissues.

Key words. αPDGF receptor, Patch, in situ hybridization, mouse embryogenesis.

Introduction

Synthesis or release of platelet-derived growth factor (PDGF) is associated with proliferation of connective tissue cell types in many pathological conditions, including atherosclerosis, oncogenic transformation and tissue response to injury. The ability of PDGF to stimulate the proliferation and migration of many connective tissue cell types in culture has been well documented (for review see Ross et al., 1986; Raines et al., 1990). Two PDGF receptor proteins, which have different affinities for the A- and B-chains of PDGF, have been identified (Yarden et al., 1986; Gronwald et al., 1988; Matsui et al., 1989). Most cultured cells derived from adult animals express high levels of the PDGF receptor β-subunit (PDGFRβ), which can bind only the B-chain of PDGF. These cells generally express only low levels of the PDGF receptor α-subunit (PDGFRα), which can bind both A-chain and B-chain and is required for the binding of PDGF-AA (Seifert et al., 1989). This lower level of PDGFRα expression is probably responsible for the relatively poor mitogenic response of most cultured cells to PDGF-AA compared with their response to PDGF-BB.

Although the PDGFRβ/PDGF-BB system seems to be the predominant player in adult animals, the possibility that the PDGFRα/PDGF-AA system may play an important role during embryogenesis was suggested by the observations that PDGFRα mRNA transcripts are detected in preimplantation mouse blastocysts (Rappolee et al., 1988) and early embryos (Mercola et al., 1990). When transcript levels were quantitated by S1 nuclease protection assay, a high level of PDGFRα was detected at the earliest period studied (E6.5), while PDGFRβ was not detectable until E7.5. This suggested that the PDGFRα might be involved in early developmental processes, but, as for virtually all of the hypotheses about the possible roles of PDGF in biological processes, this hypothesis was based largely on the proposition that expression implies function. In general, it has been very difficult to determine whether PDGF is playing a critical role in a given process, or is merely one of many growth factors making small additive contributions.
An approach to identifying those processes in development that critically depend on a particular gene is to determine the phenotype of embryos with a defective gene, and to compare the pattern of defects with the pattern of gene expression in normal embryos. The existence of the Patch (Ph) mutation, which has been shown to be a deletion of the PDGFRα gene (Stephenson et al., 1991), has permitted us to analyze this role for the PDGFRα/PDGF system. When the Ph mutation was first described, it was noted that Ph/Ph homozygotes die before birth after exhibiting certain gross abnormalities, including cleft face, subepidermal blebs, and general edema (Gruneberg and Truslove, 1960). Since it was not apparent how the defects described could be explained by a defect in PDGFRα expression, we used in situ hybridization to determine the pattern of PDGFRα expression during normal embryogenesis and performed a detailed histological search for defects in the development of Ph/Ph mutants. We report that the Ph/Ph homozygotes display a characteristic sequence of specific developmental defects that result in embryonic death at one of two stages. These defects occur in nearly all of the sites of PDGFRα expression in normal embryos including both mesodermal and neural crest derived mesenchyme. (See also Morrison-Graham et al., 1992; Orr-Urtreger et al., 1992.) These results indicate that PDGF plays a critical role in the development of many tissues and that it cannot be compensated for by other factors.

Materials and methods

Tissue preparation

C57Bl/6J × C57Bl/6J, Balb/c × Balb/c, or Patch × Patch (in either C57Bl/6J or Balb/c backgrounds) mice were mated and the date of vaginal plug detection was designated E0. Mice were anesthetized with ether and killed by cervical dislocation at various times. Embryos were removed and fixed in 0.1 M sodium phosphate-buffered 10% formalin (formalin), pH 7.0 for 1-7 days, dehydrated through graded alcohols and xylene, and embedded in paraffin. (Portions of some embryos were quick frozen in liquid nitrogen for genotyping.) For in situ hybridization, two or three embryos in one day increments from E6.5-E18.5 were collected, while two to five embryos at each of various stages from E9.5-E18 were collected for histology. All embryos were serially sectioned. 8 μm sections were cut and set aside for in situ analysis or deparaffinized and stained with toluidine blue, Harris’s hematoxylin and eosin Y, or Gomeri’s aldehyde fuchsin. (See also Morrison-Graham et al., 1992; Orr-Urtreger et al., 1992.) These results indicate that PDGF plays a critical role in the development of many tissues and that it cannot be compensated for by other factors.

Expression of PDGFRα in early postimplantation extraembryonic endoderm is not necessary for initial development

To examine PDGFRα expression and its role during early postimplantation stages, we used pluripotent ES-D3 embryonic stem (ES) cells (Doetschman et al., 1985) derived from the inner cell mass cells of the blastocyst. These cells are able to proceed in vitro through some of the stages of early embryonic differentiation. Undifferentiated ES-D3 cells do not express PDGFRα that can be detected by northern analysis, but when cultured as aggregates in suspension, they form simple embryoid bodies which resemble the inner cell mass of an E4.5 blastocyst and begin to express PDGFRα transcripts detectable by northern blot (Fig. 1). In prolonged suspension culture (>7 days), cystic embryoid bodies, consisting of a fluid-filled cavity surrounded by a cell layer with the properties of extraembryonic endoderm, start to form. This differentiation is accompanied by an increase in PDGFRα expression (Fig. 1). In simple embryoid bodies, the inner cells, which are comparable to the undifferentiated core cells of the inner cell mass of a blastocyst, rarely, if ever, express PDGFRα mRNA detectable by in situ hybridization. The outer layer of embryoid body cells differentiates into a layer with the properties of extraembryonic endoderm, which strongly hybridizes to probes for PDGFRα (data not shown). The increased expression of PDGFRα transcripts detected in northern blots of RNA from these cultures parallels the increased proportion of extraembryonic endoderm.
PDGFRα expression by embryonic mesoderm is associated with early Ph/Ph defects

At E7.5, PDGFRα expression by the extraembryonic endoderm has declined, but strong labeling can be seen in large cells in the ectoplacental cone. The embryonic mesoderm, which begins to form at around E7 is the first tissue in the embryo to express PDGFRα and is uniformly labeled at E7-7.5, while the ectoderm remains unlabeled (data not shown) (see also Orr-Urtreger et al., 1992).

As the mesoderm begins to differentiate, expression of PDGFRα mRNA becomes regionalized. By E8.5-E9 both somites and the mesenchyme are labeled, but much of the wall of the developing heart is not (data not shown). By E9.5, expression in the somites has declined. As the regional specialization of the somite proceeds, expression in the myotome becomes undetectable, but remains high in the sclerotome (Fig. 2). The level of expression of PDGFRα mRNA in the dermama appears to correlate with age; early dermama being labeled and late dermama unlabeled. PDGFRα mRNA is present throughout the mesodermal mesenchyme, including the splanchnic mesodermal plate and surrounding the neural tube and somites, as well as surrounding the developing vasculature, for example the dorsal aortae (Fig. 2). With two exceptions (described below), no ectodermal or definitive endodermal derivatives are labeled (Fig. 2).

E9.5 Ph/Ph embryos display characteristic defects, all of which can be related to the pattern of PDGFRα expression in normal embryos. Ph/Ph embryos have a kinked neural tube, subepidermal blebs flanking the neural tube, and distorted somites in regions of blebs (Gruneberg and Truslove, 1960). This reflects the virtual absence of presumptive sclerotomal cells (which express PDGFRα in normal embryos) in the vicinity of the notochord (Fig. 3), and suggests that the differentiation or proliferation of sclerotomal precursors requires PDGFRα expression. PDGFRα is also strongly expressed in normal embryos by mesenchymal cells condensing to form the major blood vessels (Fig. 2). In Ph/Ph embryos the areas around forming vessels contain fewer cells and dilation of the heart and vasculature is common in Ph/Ph embryos (Gruneberg and Truslove, 1960), possibly as a result of defective formation of the vessel wall (Fig. 3).

PDGFRα expression and the Ph/Ph phenotype in late mesodermal derivatives

During the early stages of organogenesis (E10.5-13.5), the general pattern of PDGFRα expression resembles that seen at E9.5. At E11.5 the mesenchyme is still intensely labeled, both in the head, where it is both neural crest and mesoderm derived, and in the rest of the embryo, where it is derived from mesoderm (Fig. 4). Regionalization of expression is clearly recognizable in the heart by E11.5 and in the mesenchyme by E13.5 (Figs 4, 5). (See also Morrison-Graham et al., 1992.)

(A) Mesenchyme and connective tissue

Although PDGFRα is expressed throughout the mesen-
Fig. 3. The sclerotome is deficient in E9.5 Ph/Ph embryos. Light micrographs of transverse sections through the cervical region of E9.5 normal (A) and Ph/Ph (B) embryos. Subepidermal bleb flanks neural tube and cells are absent in area flanking notocord. Neural tube (N), notocord (No), dorsal aorta (DA), bleb (Bi). Bar=100 μm.

Fig. 5. PDGFRα expression in E13.5 embryos. Sagittal section hybridized to PDGFRα antisense riboprobe. Mesenchyme (M), rib (R), dermis (D), heart (H), lung (Lu), liver (Li). Darkfield illumination. Bar=300 μm.

chyme at E13.5-15, it is particularly intense where mesenchyme surrounds developing cartilage and bone, where mesenchyme and epithelium are interacting to form new organs, and around blood vessels (Fig. 5). Corresponding to this pattern of PDGFRα expression in normal embryos, most connective tissue arising from the mesoderm in Ph/Ph embryos shows some defect by E13.5. By E18 this results in an embryo that is essentially an epidermal sac filled with organs and depleted of most connective tissue (Fig. 6). Within the organs, the connective tissue component is also often deficient. For example, most E18 Ph/Ph embryos show malformations of the esophagus and trachea and thinned or absent tendons. In some cases there are compound defects, as in the case of the esophagus, where both a disorganized smooth muscle layer and diminished submucosa are seen (Fig. 7). These abnormalities are not due to developmental delay (i.e. slow but otherwise normal development). Developmental processes which do occur, occur with normal timing, and Ph/Ph embryos do not resemble normal earlier stage embryos.

Fig. 6. Ph/Ph embryos show abnormalities in many mesodermal derivatives. Comparison of transverse sections at the level of liver and lungs in normal (A) and Ph/Ph (B) mice at E18. In the mutant mouse, the liver protrudes from the abdomen and the spinal cord is not encased by the vertebra, but is bounded dorsally by a fluid filled bleb. Liver (Li), muscle (M), vertebra (V), bleb (B), dermis (D). Bar=10 μm.

(B) Cartilage and bone
By E13.5 derivatives of the sclerotome have formed the cartilage model of the axial skeleton (spinal column and ribs) and the process of ossification is beginning. Like
Fig. 2. PDGFRα expression in E9.5 embryo. In situ hybridization using a PDGFRα riboprobe. Sclerotome (S), mesenchyme (M), roof of the fourth ventricle (R), dermomyotome (DM), heart (H), dorsal aorta (A), brain (B). Darkfield illumination. Bar=100 μm.
Fig. 4. Expression of PDGFRα in E11.5 embryos. Hybridization with (A) antisense PDGFRα riboprobe and (B) sense control probe. Sections through an E11.5 embryo. Blood is unlabeled but appears as yellowish bright spots, e.g., in the chambers of the heart, in this darkfield image. Brain (B), limb bud (Lb), sclerotome (S), heart (H), mesenchyme (M), trigeminal ganglion (T), pericardium (P), primitive trabeculae (Tr). Arrowhead in A indicates roof of fourth ventricle. Darkfield illumination. Bar = 150 μm.

Fig. 9. PDGF is important for formation of the lens. In situ hybridization of PDGFRα riboprobe to a normal E11.5 lens (L), but not to the optic cup (C) or stalk (S). Bar = 50 μm.
the sclerotome, the perichondrium/periosteum of the developing bones is strongly receptor positive. The chondrocytic core of the cartilage, however, begins to lose expression of PDGFRα transcripts (Fig. 5), and the osteoblasts which replace it express undetectable levels of transcript (data not shown). PDGFRα expression seems to be important for formation of the axial skeleton. Some early vertebral malformations and spina bifida were described in Ph/Ph embryos (Gruneberg and Truslove, 1960). At later stages of development, we find that the dorsal region of nearly all vertebral arches is absent, and that in some cases the distortions of the vertebrae are so severe that only their position allows them to be identified (Fig. 6). (See also Morrison-Graham et al., 1992.) The PDGFRα-expressing sclerotomal mesenchyme between developing vertebrae is the source of intervertebral discs. In some Ph/Ph embryos, some discs are absent, and a single vertebra spans the space normally occupied by several, apparently indicating vertebral fusion. At various stages of embryogenesis, distortions of other bones formed by endochondral ossification can also be detected, but are generally less severe than those seen in the vertebrae (data not shown). (See also Morrison-Graham et al., 1992.)

**Fig. 7.** Effect of Ph/Ph mutation on the structure of the esophagus. Toluidine blue stained sections through the esophagus of an E18 (A) normal and (B) Ph/Ph embryo. Smooth muscle (M), mesenchymal submucosa (S). Bar=30 μm.

Although expression of PDGFRα mRNA is transient in the dermatome, it is expressed throughout the dermis during development (Fig. 5). This expression is critical for proper development of skin. Ph/Ph skin is essentially devoid of a dermal layer (Figs 6, 8). A loose connective tissue web is generally seen underlying the

**Fig. 8.** Mesodermal components of skin fail to develop in Ph/Ph mutants. Skin of E18 (A) normal and (B) Ph/Ph embryos stained with Gomori's aldehyde fuchsin. Ph/Ph embryos show an unorganized epidermis (E), absence of dermis (D), loose underlying connective tissue web, and thin subcutaneous muscle in mutants. Arrowheads indicate mast cells in normal skin. (C) Normal skin at lower magnification to show the full thickness of normal skin and connective tissue above a vertebra (V). Muscle (M), stratum corneum (C). Bar=100 μm.
epidermis, and even this web is absent from skin covering blebs (Fig. 8). The epidermis is disorganized and Gomori’s aldehyde fuchsin fails to stain the stratum corneum (Fig. 8). This is consistent with studies showing dermal induction is important for epidermal proliferation and differentiation (For review see Hay, 1981, 1989). In addition, mast cells are absent or severely reduced in number in the dermis. Typically, the mesenchyme and muscle layers underlying the skin are absent as well (Figs 6, 8).

Unlike the dermis, muscle does not reinitiate PDGFRα expression as it differentiates from the myotome. Expression of PDGFRα in muscle remains undetectable in normal embryos at all stages studied. Nevertheless, Ph/Ph embryos show a complete, or nearly complete, absence of muscles of the back. At later times, additional deficits can be seen, e.g., the muscle underlying the skin is only partially formed (Figs 6, 8). It is possible that these defects result from earlier defects in the formation of the receptor-negative myotome from the receptor-positive somitic mesoderm, or from inadequate interaction of myotomal derivatives with other tissues which normally express PDGFRα.

(D) Heart
At E11.5 PDGFRα mRNA is not detected in the endocardium (Fig. 4). The myocardium is also generally unlabelled, although there appear to be small pockets of labeling in some sections. The primitive trabeculae and the pericardium are labeled (Fig. 4), as is the cushion tissue of both the atrioventricular canal and the truncus (Morrison-Graham et al., 1992). At E13.5 and later stages, PDGFRα mRNA remains undetectable in the endocardium, and in most of the myocardium (although some regions are clearly labeled; Fig. 5). At these later stages, pericardial labeling persists, and some labeling of the ventricular trabeculae can be seen (Fig. 5). By E15, rudimentary valves can be distinguished which are intensely labeled (data not shown). Severe defects are found in the hearts of Ph/Ph mutants, the most notable being the failure of the heart to septate (Morrison-Graham et al., 1992). In addition, preliminary observations indicate that valves are distorted and blood vessels of the heart less numerous (data not shown).

PDGFRα may play a role in the development of some non-mesodermal derivatives
No endothelial and only a few epithelial tissues are detectably labeled at any stage of development (Figs 2, 4, 5). This pattern of expression is consistent with studies of cultured cells which have indicated that very few, if any, cultured epithelial or endothelial cell types express PDGFRα. With only three exceptions, PDGFRα expression is not detected in any ectodermally derived tissues before E13.5, nor in the neural crest derived proximal trigeminal, sensory, or sympathetic ganglia at any age studied (see also Morrison-Graham et al., 1992).

(A) Lens
The lens is one of three ectoderm-derived structures to express detectable PDGFRα mRNA before E13.5. At E11.5, the earliest stage examined, the epithelial cells that are forming the lens of the eye express high levels of PDGFRα, while the adjacent optic cup is negative (Fig. 9). PDGFRα expression seems to be important for proper development of the lens. At a gross level, the lens of an E13 Ph/Ph embryo is distorted. In histological sections, they are seen to contain fewer fiber cells distributed in a narrower band than normal counterparts and to have voids in the lens matrix. (See Morrison-Graham et al., 1992.) Detection of expression of PDGFRα in the lens, and the defects seen in lenses of Ph/Ph mutants, partially explain the observation by Brewitt and Clark (1988) that, in organ culture, pulsatile exposure to PDGF allows the lens of the newborn rat eye to maintain transparency and normal rates of protein synthesis.

(B) Neural tube, brain and choroid plexus
PDGFRα transcripts are detected in E9 embryos in the most dorsal portion of the neural tube (data not shown). This expression is transient, and coincides with neural tube closure, and may represent labeling of early neural crest cells. Transcripts are not, however, detected before closure of the tube nor in E9.5 embryos in which the tube has completely fused, except in the roof of the fourth ventricle (Fig. 2). Both the posterior choroid plexus, which develops from this tissue (plus a mesenchymal component), and the anterior choroid plexus, express PDGFRα throughout development (data not shown). Later, at E13.5, a punctate pattern of PDGFRα expression begins to be detected in the brain, brain stem, and spinal cord which persists through adulthood (data not shown). The shape and size of receptor-positive cells suggests that they are neurons, but a more detailed analysis is required to determine whether specific nuclei or tracts are labeled.

E16 or older Ph/Ph embryos display abnormalities in the brain, but these are not consistent among specimens. For example, one specimen had a small metencephalon, another had no olfactory bulb, and another had collapsed ventricles. There may be more subtle central nervous system abnormalities, but we have not examined these in detail. Ph/Ph embryos do, however, show characteristic defects in the choroid plexus (data not shown).

Discussion
In situ hybridization demonstrates that the PDGFRα is expressed more widely during embryogenesis than we had anticipated, and that the pattern of expression often changes dramatically during the course of formation of organs and tissues. Fig. 10 summarizes the pattern of PDGFRα expression beginning from the three germ layers. (For details on neural crest expression, see Morrison-Graham et al. (1992).) The endoderm and its derivatives never express PDGFRα,
the ectoderm is generally negative until relatively late in development, when expression becomes detectable in a few derivatives. The mesoderm expresses PDGFRα early in development and shows the most complex pattern of regulation of expression. It is positive at the time it is first formed, but expression is transiently or permanently reduced during the differentiation of several of its derivatives. For example, the somites derive from receptor-positive mesenchyme but by the time that regions of the somite have differentiated into the sclerotome, myotome and dermatome, the cells in the myotome and late dermatome no longer express detectable receptors. Derivatives of the myotome remain receptor-negative during the formation of skeletal muscle, but derivatives of the dermatome begin to re-express PDGFRα during the formation of the dermis. Derivatives of the receptor-positive sclerotome, in contrast, continue to express PDGFRα until they have differentiated into chondrocytes, at which time PDGFRα expression is no longer detected.

The observation that all Ph/Ph embryos die before birth suggests that PDGFRα is playing an important role during development. By determining the location and timing of abnormalities in the development of Ph/Ph embryos and comparing this with the pattern of expression of PDGFRα during normal development, we have attempted to determine the nature of the involvement of the PDGFRα in development. Lack of PDGFRα expression does not seem to affect the formation of the mesoderm layer, suggesting that the receptor is not required for mesodermal differentiation, but is induced as a result of it. However, at later stages of development, virtually all mesodermal derivatives are affected by the absence of the receptor. There seems to be a direct relationship between expression of PDGFRα and the defects seen in Ph/Ph embryos. Cell types which would be receptor-positive in a normal embryo are present in reduced numbers in Ph/Ph embryos, sometimes resulting in the virtual elimination of a component of an organ. Some of these proposed causal links are discussed below. It should be noted at this point that although c-kit, the nearest gene for which a probe is available (Chabot et al., 1988; Geissler et al., 1988) is not affected, the ends of the Patch deletion have not been localized to within the PDGFRα gene and it is possible that the Patch deletion might affect an additional gene. Nevertheless, the correspondence between the pattern of PDGFRα expression and the defects seen in Patch mice argues that the lack of the receptor is the primary cause of defects in the mutants.
The Ph/Ph embryos examined (which have been verified as Ph/Ph by Southern blotting) do not display any defects which cannot be simply explained as results of deletion of the PDGFRα.

Ph/Ph mutant embryos are substantially smaller than normal littermates (about 60% of normal weight). This probably at least partially reflects the net effect of the near complete lack of some cell types, e.g. the sclerotomal cells, and a significant reduction in the numbers of other receptor-positive mesodermal derivatives. In addition, some of the growth retardation could also result indirectly from changes in development of extraembryonic support tissues. The placenta expresses high levels of PDGFRα mRNA, and the yolk sac is moderately labeled in the region proximal to the chorion (data not shown). In the one yolk sac examined, fewer blood islands were found than in normal animals, and an abnormal papillary structure was seen. (See also Orr-Urtreger et al., 1992.)

Most of the specific defects that are visible by E9.5 appear to be related to the lack of sclerotomal cells. The absence of these cells and of the extracellular matrix which they normally secrete, probably results in the development of blebs, edema, and swelling of the aorta by straightforward physical processes. It is also possible that the distortion of the neural tube results from a growth imbalance between the sclerotome and the neural tube.

Ph/Ph embryos that survive beyond E9.5 seem to enjoy a brief respite: the severity of observable defects actually decreases temporarily (Morrison-Graham et al., 1992). The subepidermal blebs regress, and some sclerotomal cells do eventually develop and form approximations of normal structures. This suggests that although PDGFRα is important during the early period, in some embryos its absence can eventually be compensated for, albeit temporarily and less efficiently, by the function of some other growth regulatory system.

Following this period of diminished severity of defects which ends around E12, Ph/Ph embryos begin to show abnormalities in virtually all derivatives of the mesoderm and some defects in ectodermal derivatives (Figs 6-8). Many mesodermal derivatives are absent or diminished in size. While all major defects detected are confined to the mesoderm (and PDGFRα expressing ectoderm), subtle changes in ectodermal derivatives also result which suggest an inappropriate inductive interaction with the mesenchyme. These “inductive” defects in ectodermal derivatives which do not express PDGFRα, such as those seen in the epidermis, are much less severe than those observed in PDGFRα expressing ectoderm e.g. in the lens and choroid plexus.

Cell culture studies have indicated that PDGF may play a role late in development in regulation of the proliferation and differentiation of glial cells (for review see Raff, 1989). PDGF expression has been detected in neurons in the developing brain (Sasahara et al., 1991; Yeh et al., 1991). These findings suggest that PDGF may play a role in the development and/or function of the brain. Localization of PDGFRα mRNA in the brain at E13.5, just prior to the time at which PDGF is first detected, indicates that the target(s) for the brain PDGF may also be in the brain. The PDGF/PDGF receptor system may have little or no role in the initial establishment of the neural ectoderm, but it may be important at the time of closure of the neural tube and again late in embryogenesis and after birth. Four E16-18 Ph/Ph embryos exhibited no characteristic gross defects in the brain, but abnormalities were apparent in all of the brains. It is difficult to use the Ph/Ph embryos to obtain information about the nature and cause of these defects, or about the role of PDGFRα expression in the putative neurons, since most of the mutant embryos have died by the time PDGFRα expression in the brain of normal embryos is well established.

We wish to thank Alayne Cartwright and Ali Sarram for technical assistance and Tom Barrett and Ron Seifert for critical reading of the manuscript. This work was funded by NIH grants GM-35501 and HL-03714 and was carried out while D. B.-P. was an Established Investigator of the American Heart Association, J. A. W was funded by PHS Grant DE-04316 and K M G. by an American Heart Association, Oregon Affiliate, Fellowship. During the preparation of this paper, J. A. W. was a Meyerhoff Visiting Professor at the Weizmann Institute of Science, Rehovot, Israel.

References


(Accepted 10 February 1992)